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Physicochemical properties and structure of starches extracted from raw, baked and boiled *Coulaedulis* (Bail.)seeds

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Key words: Cooked seed, *Coulaedulis*, Physicochemical property, Starch hydrolysis, Starch seed.

Abstract

The aim of the present study was to investigate the proximate composition and physicochemical properties of starches extracted from raw ($SEFCeS_{ra}$), boiled ($SEFCeS_{bo}$) and baked ($SEFCeS_{ba}$) *Coulaedulis* seeds. The cooking treatment did not have any profound effect on moisture, ash, protein and crude fiber contents of starches extracted from seed, except for the fat content. Starch purities were high (97.40%) with low protein (0.49%), fat (1.66-1.92%), crude fiber (0.03%) and ash (0.20%) contents. Granules of $SEFCeS_{ra}$ were round in shape, with sizes ranging from 4.29-21.39 µm. They did not show unusual features of granules such as collapsed granule, cracked surface and some defects, but those of $SEFCeS_{bo}$ and $SEFCeS_{ba}$ showed critical morphological changes. The loss of starch granules integrity was greater in the boiled seeds. At temperatures between 70 and 90°C, $SEFCeS_{ra}$ had the highest and $SEFCeS_{bo}$ had the lowest swelling power. Solubilities of SEFCeSra, $SEFCeS_{bo}$ and $SEFCeS_{ba}$ increased as the temperature increased from 50 to 95°C. Percentage transmittance values of the starches extracted from *Coulaedulis* seeds increased (42 to 52%) after cooking. The value of $SEFCeS_{bo}$ was higher than that of $SEFCES_{ba}$. In all the starches, percentage transmittance was slightly reduced as the number of weeks of storage increased. The relative order of hydrolysis was $SEFCES_{bo}$>$SEFCES_{ba}$>$SEFCES_{ra}$. Distinct differences of physicochemical properties were observed between starches extracted from $SEFCeS_{ra}$, $SEFCeS_{bo}$ and $SEFCeS_{ba}$. They may have broad possibilities as ingredients in food systems and other industrial applications.

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Introduction

Coulaedulis Bail. is a member of the family Olacaceae that comprises 250 species (Mabberley, 1997). It is an evergreen tree growing to a height of 25-38 m. The tree can be found in the top canopy of forests as well as the lower story and has no special soil requirements (Tamokou *et al.,* 2011). *C. edulis* is a commonly occurring medicinal plant in Africa. The stem and fruits of this plant are commonly used in West Africa for the treatment of stomach ache and skin diseases. It has shown antibacterial and anti-yeasts activities (Adebayo-Tayo and Ajibesin, 2008; Tamokou *et al.,* 2011) and is used as tonifiant (Iwu, 1993). The bark of *C. edulis* is used for dressing sores, to treat dysentery, to stimulate appetite (Duke, 2001) and to produce rinses or enemas for loin pains or kidney problems. It is also reported to contain acetylenes known to exhibit anticancer activity (Dembitsky, 2006). The fruit of *C. edulis* is a seasonal product, which is produced in the period from January and May (Alan, 1999). It is described as a nut, ellipsoidal in shape, being about 3-4 cm long with flesh 5-6 mm thick surrounding the kernel (Alan, 1999). *C. edulis* is commonly known as African walnut or Gabon nut tree due to its edible seeds (Adebayo-Tayo and Ajibesin, 2008). In Côte d'Ivoire, *C. edulis* seed is one of the seeds which is neglected, underutilized, underdeveloped and even going into extinct. However in Cameroon and Nigeria, it's used as food components (Tchiegang *et al.,* 1998). As the seeds have a high humidity, they can be contaminated easily by mushrooms during the stockpiling, hindering its commercialisation. Fresh seeds of *C. edulis*contain 34.9% of fat and 38.6% of starch, while the raw flour contains 33.9% and 44.1% respectively and 604 mg/100 g of potassium and 393 mg/100 g of phosphorus (Ekissi *et al.,* 2005). Fatty acids containing a large proportion of oleic acid (95.5-97.4%) and triacylglycerides were found in the oil (Tchiegang *et al.,* 1998). Seeds are generally consumed after various processes like soaking, cooking, milling, roasting, puffing and germinating (Güzel and Sayar, 2011). However, soaking followed by cooking is the most common way of the production of edible seed products. Soaking is carried out below the gelatinization temperature and intended for increasing the water content to accelerate the following cooking step (Güzel and Sayar, 2010). Cooking is done above the gelatinization temperature for gelatinizing starch and to produce a tender edible product, to develop aroma and to improve the overall acceptability of the seeds (Chavan *et al.,* 1986; Tharanathan and Mahadevamma, 2003). These processes can cause more reductions in minerals levels (Nestares *et al.,* 1999), protein quality by destruction or inactivation of the heat-labile antinutritional factors (Chau *et al.,* 1997; Wang *et al.,* 1997; Vijayakumari *et al.,* 1998) and alter the physicochemical properties of native starch to improve functional characteristics (Chen *et al.,* 2003). However, very limited information is available about the effect of cooking methods on the biochemical composition and physicochemical characteristics of starches in *C. edulis* seeds.

The objective of this work is to study the proximate composition and physicochemical properties of starches extracted from raw, baked and boiled *C. edulis* seeds (baking and boiling are used in preparing different foods in many countries). An acquisition of understanding of properties of these starches may demonstrate its further potential uses in the food industry as an alternative source to conventional forms of starches.

Materials and methods

Materials and chemicals: Fruits of *C. edulis* used for this work were randomly harvested at maturity from a farm in Azaguié, South-East portion of Côte d'Ivoire (West Africa) in February 2009. The climate in this area is characterized by high humidity, precipitation up to 4,000 mm per annum and relatively high temperatures, averaging 27°C. The raw materials were physically examined to ensure disease-free. Then, they were immediately transported to the Laboratoire de Biocatalyseet des Bioprocédés and identified with the help of experts at the Université Nangui Abrogoua (Abidjan, Côte d'Ivoire). These fruits were stored under prevailing tropical ambient conditions (25°C, 60-85% RH) for 24 h and carefully

cracked. The raw seeds removed were cleaned of any adhering residue and hand-picked to eliminate damaged ones. The selected seeds were stored until further use at -20°C. The snails *Archachatinaventricosa* were purchased from a local market (Abidjan, Côte d'Ivoire) and left fasting for three days at ambient temperature (25°C) before enzymatic solution preparation. All the chemicals, reagents and solvents used in the experiments were of analytical grade and were products of Sigma Chemical Co. (St. Louis, MO).

Cooking treatment

Prior to heat processing, selected seeds were slit with a sharp knife and washed several times with deionised water. They were dried at ambient temperature (25°C) and separated in three groups of similar weight (approximately 1.5 kg). The first was placed inside the boiling water (seed:water, 1.5:2, wt/wt) and was allowed to boil (98±1°C) for 30 min on a hot plate. The water was drained off after boiling and the hot samples were exposed to the air to allow surface water to evaporate for 1 h. The second was placed single layer in a sealed aluminium pan and baked in an oven at 160°C for 30 min. Then, the hot samples were treated as described above. The third (raw seeds used as control) did not undergo heat treatment. Isolation of starch: Starches from raw, baked and boiled seeds were isolated by a method modified from the method of Delpeuch *et al*. (1979). The *C. edulis* seeds (raw and cooked) were washed several times with deionised water to completely remove any traces of alkali on the seed surfaces. They were then wet ground in a blendor (Lyon-France) with deionised water (1:1, seed to water) for 10 min and the homogenate was passed sequentially through 500, 250, 125 and 100 meshes Tyler screens. The extract was allowed to settle overnight at refrigerated temperature (4°C). The supernatant was decanted. The starch pellet was dispersed in 1.5 l of 4% (w/v) NaCl with stirring at regular intervals and allowed to settle for 2 h at refrigerated temperature (4°C) in order to remove any protein fractions adhering to the starch granules. The supernatant was decanted and the sedimented starch was resuspended in 1.5 l of deionised water. The starch suspension was allowed to sediment and the supernatant was discarded again. The process of resuspension in deionised water and sedimentation was repeated 7 times, until the settled starch gave a firm, dense deposit. The starch was washed twice with 80% (v/v) ethanol and dried overnight at 45°C in an air oven (MMM MED center) for 48 h. It was ground in mortar, passed through a 100 mesh screen and stored in sealed glass jars at room temperature (25°C) until further use.

Proximate composition analysis

The dry matters contents were determined by drying in an oven at 105°C during 24 h to constant weight (AOAC, 1990). The crude protein contents were calculated from nitrogen contents (Nx6.25) obtained using the Kjeldahl method by AOAC (1990). The total ash contents were determined by incinerating in a furnace at 550°C (AOAC, 1990). The crude fat contents were determined by continuous extraction in a Soxhlet apparatus for 8 h using hexane as solvent (AOAC, 1990). The crude fibre contents were determined according to the AOAC (1990) method. The reducing sugar contents were determined according to the method of Bernfeld (1955) using 3.5 dinitrosalycilic acid. The total starch contents were determined as reducing sugars after complete acid hydrolysis and calculated as glucose x 0.9 (AOAC, 1997).

Spectrophotometry of iodine-starch complexes

Iodine spectra of the starch was determined by method of Van Hung and Morita (2005). Starch sample (100 mg) was dispersed in 10 ml of 10%-urea containing dimethyl sulphoxide (90% DMSO, 10% 6 M urea) by vigorous vortexing and heated in a boiling water bath for 30 min. The dispersion was centrifuged at 5000 g for 15 min and cooled to room temperature for 20 min. Aliquots (1 mL) of the dispersion were transferred to a 25 ml volumetric flask, mixed with about 10 ml of deionised water and 0.30 mL of iodine-potassium iodide (200 mg I2 and 2 g KI in 100 mL deionised water) solution. The mixture was stood for 20 min at room temperature and an absorption curve was measured from 400 to

700 nm with a spectrophotometer (JASCO V-530 UV/VIS, MODEL TUDC 12 B4, JAPAN SERVO CO. LTD INDONESIA). The wavelength at maximum absorption (λmax) and absorbances at 630 nm (blue value) and at λmax were determined according to Fujimoto *et al.* (1972) method.

Granular morphology

Starch samples were suspended in ethanol to obtain a 1% suspension. One drop of the starch-ethanol solution was applied to an aluminium stub using double-sided adhesive tape and the starch was coated with gold–palladium (60:40). An accelerating potential of 10 kV was used during micrography (Kaur *et al.,* 2004).

Particle size distribution

Particle size distribution of starch was done using a Coulter small volume module model LS230 laser light scattering particle size analyser. 0.25 g of starch sample was combined with 3 mL of deionised water in a small glass vial and vortexed, followed by sonication for 1 h. Dried sample was completely deagglomerated after approximately 10 min of sonication at 40°C. The sample was vortexed and approximately 10 drops were added to the sample port until the instrument read 45% Polarization Intensity Differential Scattering (PIDS) or 10-14% obscuration. Isopropanol was used as the suspension fluid within the instrument. The sample was allowed to equilibrate the isopropanol for 15 min before starting the analysis (Kaur *et al.,* 2004, 2011).

Swelling power and water solubility

The water solubility and swelling power were carried out using the methods described by Leach *et al.* (1959) with some modifications. Starch (100 mg, dry weight basis) was weighed directly into a screw-cap test tube and 10 mL deionised water was added. The capped tubes were then placed on a vortex mixer for 10 s and incubated in 50, 55, 60, 65, 70, 75, 80, 85, 90 and 95°C water bath for 30 min with frequent mixing by vortex at 2 min intervals. The tubes were then cooled to room temperature in an iced water bath and centrifuged at 5000 g for 15 min and the supernatant was removed with suction. The cloudy solid layer was considered as supernatant, only the material adhered to the wall of the tube was thought as sediment and weighed (Ws). The supernatant and sediment were dried to constant weight in a forced-air oven at 105°C for 24 h and weighed. The Water Solubility (WS) and Swelling Power (SP) were calculated as follows:

$WS = (W1/0.1) \times 100\%$

$SP = (Ws-W2) \times 0.1$

where, W1and W2 were weight of supernatant and centrifuged swollen granules, respectively.

Gel clarity behaviour during storage at 4°C

Starch gel clarity was determined according to the method of Craig *et al.* (1989). A 1% aqueous suspension was made by suspending 0.2 g (db) starch in 20 ml of distilled water in a stoppered centrifuge tube and vortex mixed. The suspension was heated in a boiling water bath for 30 min thoroughly shaking every 5 min. After cooling at ambient temperature for 10 min, the clarity of starch was determined by measuring percent transmittance at 650 nm against water blank on a spectrophotometer (JASCO V-530 UV/VIS, MODEL TUDC 12 B4, JAPAN SERVO CO. LTD INDONESIA). The other stoppered centrifuge tubes were conditioned at 4°C for 4 weeks. After every week, the clarity of starch was determined by measuring percent transmittance at 650 nm.

In vitro digestibility

The digestive juice of the snails (*Archachatina-ventricosa*) was collected according to the method described by Colas (1977). Snails were left fasting for three days and then their shell was carefully broken. The digestive tracts were isolated and the coloured brown juice was collected. This digestive juice was centrifuged at 10,000 g for 15 min at 4°C. The supernatant filtered through cotton wool was used as the enzymatic solution. It contained alpha-amylase and alpha-glucosidase activities (Colas and Attias, 1975; Colas and Attias, 1977; Soro *et al.,* 2007; Cissé *et al.,* 2013). Starch sample (500 mg) was incubated with the enzymatic solution (200 µL, 20 U) in 10 mL of a 0.1 M sodium acetate buffer, pH 5.0, in a shaking water bath at 37°C. Aliquots (0.1 mL) from the

reaction mixture and control were separately withdrawn at time intervals between 0 and 160 min. Each aliquot was immediately mixed with 0.2 mL of DNS to arrest further enzymatic activity. The reducing sugars (glucose) were estimated at room temperature (25°C) by the Bernfeld (1955). The reducing sugar produced was calculated from a standard curve and its time course plotted on another graph.

Statistical analyses

The mean values and standard deviations of each analysis are reported. Analysis of variance (ANOVA) was performed as part of the data analyses (SAS, 1989). When F-values were significant ($p<0.05$) in ANOVA, then least significant differences were calculated to compare treatment means.

Results and discussion

Proximate composition

The proximate composition of starches extracted from raw ($SEFCeS_{ra}$), baked ($SEFCeS_{ba}$) and boiled ($SEFCeS_{bo}$) *C. edulis* seeds were presented in Table 1. The purities of all starches were very high (>97%) with no significant differences among them ($p>0.05$). This result seems that the methodology employed for the isolation of starches from *C. edulis* seeds leads to pure starch. Therefore, these starches are qualified for physicochemical characterization. Total starch contents in the starches extracted from *C. edulis* seeds (SEFCeS) was similar to those of potato (97%) and cassava (97%) starches (Bertolini *et al.,* 2005). However, this rate was higher than those reported for different haricot bean (Phaseolus vulgaris, 95-96%, Shimelis *et al.,* 2006) and andean bean (Pachyrhizusahipa, 56-59%, Bertolini *et al.,* 2005) varieties. It can be seen form Table 1 that the protein, crude fibre, moisture and ash contents of $SEFCeS_{ba}$ and $SEFCeS_{bo}$ were similar ($p>0.05$) with that of the control ($SEFCeS_{ra}$), except for the fat content ($p<0.05$). These components are present in SEFCeSbecause of the difficulty of separating highly hydratable fine fibre during the wet fractionation process and the strong adherence of insoluble protein to the starch (Otto *et al.,* 1997). The ranking of the fat level was $SEFCeS_{ra}>SEFCeS_{ba}>SEFCeS_{bo}$, indicating that the samples showed a decrease in fat level and amylose-lipid complexes during the cooking. This result is very important that the amylose-lipid complexes affect swelling and solubility (Morrison *et al.,* 1993; Fredriksson *et al.,* 1998). Morrison (1995) showed that lipid-complexed amylose reduced swelling power, while lipid-free amylose facilitated swelling. This finding seems that amylose acts as diluent and inhibitor of swelling, especially in the presence of lipids which can form insoluble complexes with amylose during swelling (Leach *et al.* 1959). The moisture content has been proved as an inherent physicochemical property of starch granule, which is influenced by the crystalline structure of starch granule (Wang *et al.,* 2006). In addition, the moisture content of starches is relevant to the environment temperature, humidity and the moisture penetrability of the package bag (Zhou *et al.,* 2013). The 8.39% moisture level of starches from *C. edulis*seeds observed here was lower than the 10.02%, 10.9-11.7% and 10-20% moisture levels reported for *Antiarisafricana*seed starches (Nwokocha *et al.,* 2012), soybean seed starches (Stevenson *et al.,* 2006) and commercial starches (Soni *et al.,* 1993), respectively. Moisture content of all the starch samples was below 9%, thereby giving the starches a better shelf life (Aryee *et al.,* 2006). Starch proteins occur on the surface (and include non-starch derived proteins) and regardless of origin are embedded within the matrix of granules. It also has the potential to moderate starch functionality (Appelqvist and Debet, 1997). The results showed that the protein content of SEFCeS was 0.49±0.01%. This value was lower than the 0.53, 0.64, 0.65 and 0.89-0.97% reported for cowpea (*Vignaunguiculata*, Chung *et al.,* 1998), red cocoyam cormel (*Colocasiaesculenta*, Awokoya *et al.,* 2012), white cocoyam cormel (*Colocasiaantiquorum*, Awokoya *et al.,* 2012) and bean (*Phaseolus vulgaris*) varieties grown in East Africa (Shimelis *et al.,* 2006) respectively. This finding is very important that starches extracted from *C. edulis*seeds (SEFCeS) could be used in the manufacture of high-glucose syrups as this protein level was similar with FDA protein content limits for

corn starch (0.4%) used for this purpose (Torruco-Uco and Betancur-Ancona, 2007). It is also very interesting to observe that the lower protein level suggests that the SEFCeS could not turn black during their storage (Ratnayake *et al.,* 2002; Tyler *et al.,* 1981). Also, this lower protein level could not have any influence on the digestibility and swelling power of SEFCeS (Schoch and Maywald, 1968). These hypothesis could be supported by the observations of Eerlingen and Delcour (1995) who speculated that high contents of proteins may have an impact on Resistant Starch (RS) type I (physically inaccessible starch) rather than on RS type III (retrograded starch).

Starch and protein interact due to attraction of their opposite charges and form inclusion complexes during gelatinization and this restricts swelling. The total ash content (0.20±0.04%) was higher compared to those of starches from different cassava varieties (0.01-0.19 %, Charoenkul *et al.,* 2011), maca root (Lepidiummeyenii, 0.12±0.01%, Rondán-Sanabria and Finardi-Filho, 2009) and lower than observed for achira rhizomes (*Canna indica*cv BS, 0.53% andrade-Mahecha *et al.,* 2012). The result in the present study indicates that starches extracted from *C. edulis*seeds originated from Côte d'Ivoire had lower crude fibre content (0.02±0.01%) than starches from maca root (*Lepidiummeyenii*, 0.2%, Rondán-Sanabria and Finardi-Filho, 2009) *C.esculenta*corms originated to Nigeria (0.20-1%, Nwanekezi *et al.,* 2010; Alinnor and Akalezi, 2010).

Spectrophotometry of iodine-starch complexes

The amylose content in starches has an important effect on their functional properties. Therefore, it is quite important that the amylose content be quantified for food processing and quality. However, literature has pointed out a controversy related to amylose determination (Martinez and Prodolliet, 1996). So, in this study, we decided to determine the wavelength at maximum absorption (λmax) and absorbances at 630 nm (O.D630 nm, blue value) and 540 nm (O.D540 nm)of each starch sample. These quality characteristics will enable us to understand the effect of seed cooking on the amylose and amylopectin degradations. The results obtained were shown in Table 2. The λmax for $SEFCeS_{ra}$, $SEFCeS_{bo}$and $SEFCeS_{ba}$were similar ($p>0.05$) (540 and 630 nm). This means that these starches contained amylopectin and amylose. The absorbances at 540 nm of starches extracted from cooked *C. edulis* seeds were significantly different ($p<0.05$) from that of $SEFCeS_{ra}$. The blue value was higher for $SEFCeS_{ba}$ (0.110 ± 0.05) than that for $SEFCeS_{bo}$ (0.090±0.04) and these were significantly different ($p<0.05$) for the two starches. This implies that the amylose content of $SEFCeS_{ba}$ was higher than that reported for $SEFCeS_{bo}$ indicating that boiling has degraded starch in *C. edulis* seed than baking. These findings were confirmed by the O.D630 nm/O.D540 nm ratios (Table 2).

Morphological characteristics

Scanning electron microscopy (SEM) images of starches extracted from raw and cooked *C. edulis* seeds were shown in Fig. 1 and 2. The $SEFCeS_{ra}$ morphology was significantly different from that of $SEFCeS_{bo}$ and $SEFCeS_{ba}$. Evidently, the micrographs of $SEFCeS_{ra}$ (Fig. 1 and 2A) did not show unusual features of granules such as collapsed granule, cracked surface and some defects, indicating the intactness of the extracted starch sample.

This demonstrates that the methodology used to obtain the starch from raw *C. edulis* seeds is effective and doesn't alter the morphology of the granules. This can be attributed to the use of simple physical operations such as drying, grinding and sieving, in which changes to the morphology of the granules may be minimal as compared to other methodologies that involve pH modification and/or extraction using chemical reagents like hydrochloric acid and sodium hydroxide (Andrade-Mahecha *et al.,* 2012). The granules of $SEFCeS_{ra}$ showed the presence of pores (Fig. 1) as reported earlier by Huber and BeMiller (2000) and Benmoussa *et al.* (2006). By SEM investigations, the $SEFCeS_{ra}$ molecules are round in shape (Fig. 1 and 2A), with sizes ranging from 4.29-21.39 μm (Fig. 3). This result agrees with those found

by Mweta *et al.* (2008) and differs for starches from rice cultivars grown in Nigeria (Ashogbon and Akintayo, 2012), yam (*Dioscoreaezingiberensis*, Zhou *et al.,* 2013) and achira (*Canna indica*andrade-Mahecha *et al.,* 2012). The mean granule diameter was 10.94 mm. In the SEM images, $SEFCeS_{bo}$ (Fig. 2B) and $SEFCeS_{ba}$ (Fig. 2C) showed critical morphological changes. The starch granules of baked seeds were obviously less degraded compared to starch granules of boiled seeds. Indeed, these starch granules were almost completely degraded. Marconi *et al.* (2000) explained that this morphological change was due to the cell wall being broken and shattered during cooking. The degree of morphological changes during the thermal process varies according to the cooking methods tested (Aguilera *et al.,* 2009).

During this thermal process, the starch granules gelatinize, swell and disrupt (leaching of amylose) and lose their crystalline structure. This phenomenon could contribute to the increase of damaged starch in cooked *C. edulis* seeds. Starch gelatinization is a complex process where the structure and functionality of the end product vary depending on process conditions such as temperature, moisture and the presence or absence of shear. These results indicate that cooking altered the granular structures of *C. edulis* starch, thereby requiring less time to hydrate and disrupt the remaining starch structures during subsequent cooking.

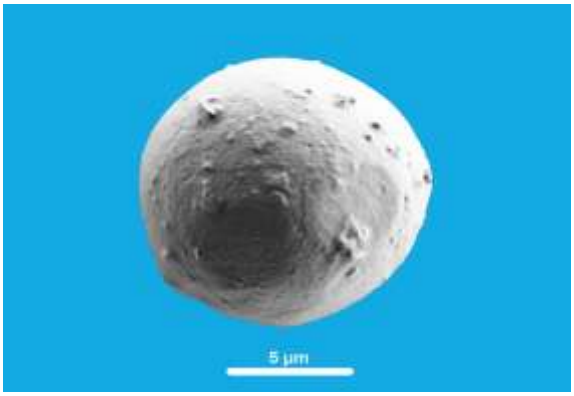
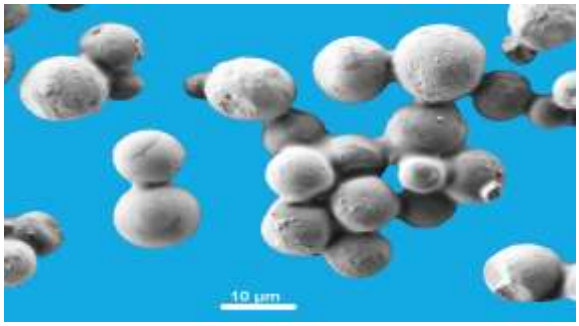
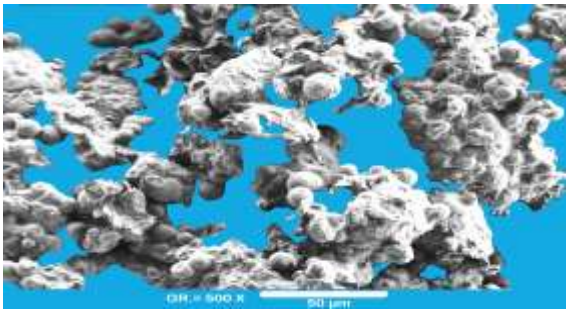


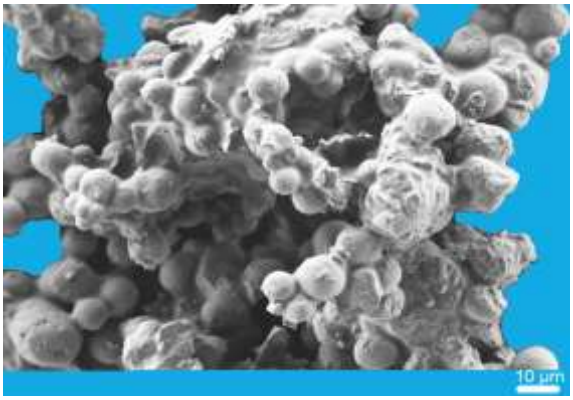
Fig. 1. Scanning Electron Microscopic Photomicrograph of a whole granule of starch extracted from raw *Coulaedulis* seeds.



a



b



c

Fig. 2. Scanning Electron Microscopic Photomicro-graphs of starches extracted from raw (A), baked (B) and boiled (C) *Coulaedulis* seeds.

Swelling power and solubility

Swelling power indicates the water holding capacity of starch, which has generally been used to demonstrate differences between various types of starches (Crosbie, 1991). The swelling power (SP) of $SEFCeS_{ra}$, $SEFCeS_{bo}$ and $SEFCeS_{ba}$ increased with increasing temperatures then decreased after further increasing temperature to 95°C (Fig. 4). At this stage, the starches were ruptured and started to disperse, allowing more leaching of soluble materials and resulting in decreased swelling power (Noranizan *et al.,* 2010). At temperatures below 60°C, the swelling power of all samples was lower than 2 g H_2O/g

sample. When the temperature was raised to 65°C, the SP of starches increased dramatically to 23 g H2O/g sample. Starch samples had similar swelling power at temperatures between 50 and 70°C. At temperatures between 70 and 90°C, the swelling power of starches of cooked seed was lower in all cases as compared to native starches. $SEFCeS_{ra}$had the highest and $SEFCeS_{bo}$ had the lowest swelling power (Fig. 4). Reduction in swelling power after cooking is attributed to structural disintegration within the granules of the starch during the process ofmodification. When starch is heated in the presence of water, the crystalline structure is transformed toan amorphous structure. Exposed hydroxyl groups of amylose and amylopectin form hydrogen bonds with water molecules. This interaction triggers granule swelling. On the other hand, the interactions among glucan chains retain the structural association originated from the granular structure. The equilibrium between granule swelling and retaining governs the swelling power. A number of factors may affect such equilibrium, which include the ratio and fine structure of amylose and amylopectin, granule-bound protein and lipid compounds and thermal treatments (Simsek *et al.,* 2009). Between 70 and 90°C, the swelling powers of $SEFCeS_{ra}$, $SEFCeS_{bo}$ and $SEFCeS_{ba}$was between those of cassava starch and corn starch, reaching 23 g H2O/g sample at 90°C, as compared to 58.8 g water/g starch for cassava starch and 16.76 g water/g starch for corn starch (Betancur-Ancona *et al.,* 2001). The high swelling of the *C. edulis*starch make it a potential additive in sausagetype meat products, as these properties are essential for proper texture in these foods (Carballo *et al.,* 1995). Solubility is the percent amount of starch leached out into the supernatant in the swelling volume determination (Singh *et al.,* 2005). It was studied to understand the nature of intra-granular bonds. The water binding capacity in commercial starches is important to the quality and texture of some food products because it stabilize them against effects such as syneresis, which sometimes occurs during retorting of freezing (Baker *et al.,* 1994). Solubility of $SEFCeS_{ra}$, $SEFCeS_{bo}$ and $SEFCeS_{ba}$ increased as the temperature increased from 50 to 95°C (Fig. 5). This could indicate that $SEFCeS_{ra}$, $SEFCeS_{bo}$ and $SEFCeS_{ba}$ are not gelatinized completely under these test conditions. Therefore, these starch samples continued to dissolve into water. Solubility of native starch was higher compared to starches of cooked seed (Fig. 5). Gujska *et al.* (1994) reported a notable increase in solubility for pinto bean, navy bean and field pea starches, beginning at 70°C, because the swollen starch granules allow amylose exudation. This is similar to the behaviour of starch from *C. edulis* seed (Fig. 5), but differs from the rise in corn starch solubility beginning at 60°C. The maximum value of 35% was observed at 95°C compared with 32 and 30% of $SEFCeS_{ba}$ and $SEFCeS_{ra}$, respectively. They were lower than that for cassava (53.8%) but higher than that for corn (15.8%) at the same temperature (Betancur-Ancona *et al.,* 2001). It is believed that the presence of weak intra granular binding and loose linking of the linear amylose fraction with the rest of the macromolecular structure contributes to high solubility (Soni *et al.,* 1987). Plots of swelling power vs. solubility of $SEFCeS_{ra}$, $SEFCeS_{bo}$ and $SEFCeS_{ba}$ were shown in Fig. 6. For these starches, it can be seen that the solubility increases with the swelling power. This could be explained by assuming that a part of linear component is involved in micellar network, while the rest is free from enlargement and is able to leach out after hydration (Soni and Agarwal, 1983). This result is similar to that reported for corn starch.

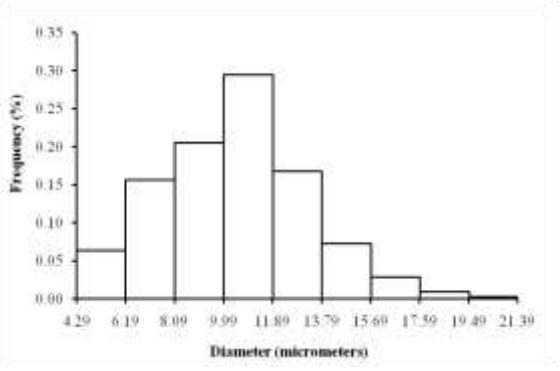


Fig. 3. Diameters distribution of starch granules extracted from raw *Coulaedulis* seeds.

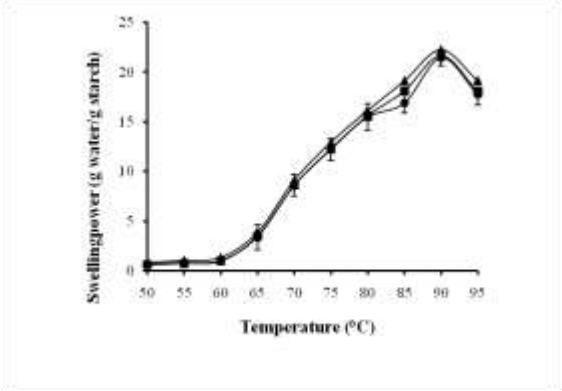


Fig. 4. Swelling power of starches extracted from raw (●), baked (¦) and boiled (▲) *Coulaedulis* seeds.

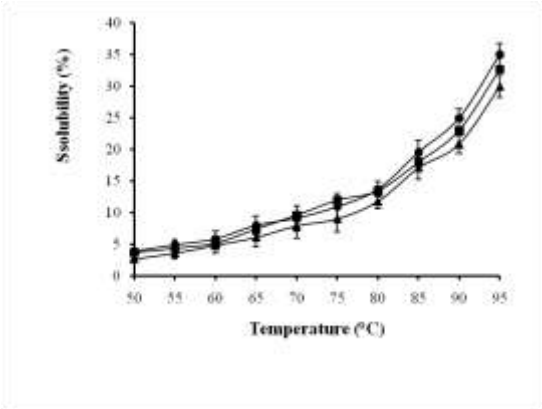


Fig. 5. Solubility of starches extracted from raw (●), baked (¦) and boiled (▲) *Coulaedulis* seeds.

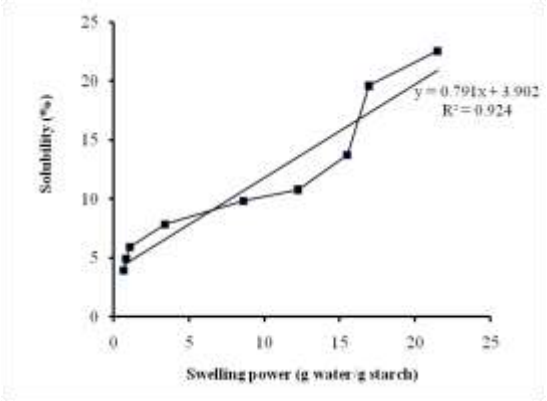


Fig. 6. Swelling power vs solubility of starches extracted from raw (●), baked (¦) and boiled (▲) *Coulaedulis* seeds.

Gel clarity behaviour during storage at 4°C

Clarity is a key parameter in starch paste quality because it gives shine and opacity to product colour. Percentage transmittance values of the starches extracted from *C. edulis* seeds increased (42 to 52%) after heating (Fig. 7). Increase in % transmittance after cooking is due to retrogradation tendency. This phenomenous is minimised in $SEFCeS_{bo}$ and $SEFCeS_{ba}$, because such associative bonding forces, responsible for retrogradation, have been reduced by the introduction of other functional groups. The value of $SEFCeS_{bo}$ was higher ($p<0.05$) than that of $SEFCES_{ba}$ and showed them to be more translucent than commercial corn starch (22.4%) (Betancur-Ancona *et al.,* 2001) and but significantly less than cassava starch (51.8%, Torruco-Uco and Betancur-Ancona, 2007). Hoover *et al*. (1996) comment that starch paste degree of transmittance is directly affected by degree of swelling. This coincides with the present results in that the *C. edulis* seed (raw and cooked) starches had greater swelling power and consequent higher clarity in their paste, than had corn starch. The *C. edulis* seed (raw and cooked) starch paste's higher transmittance is probably because their amylose contents have greater clarity (Swinkels, 1985). The clarity of a starch gel directly influences the shine and colour of products that contain it as a thickener (Betancur-Ancona *et al.,* 2003, 2004). That why, the *C. edulis* starch's excellent clarity makes it potentially useful in sauces (Chel-Guerrero and Betancur, 1998) and in products such as fruit pie fillings and candies (Torruco-Uco and Betancur-Ancona, 2007).

The influence of storage weeks on paste clarity of starches from *C. edulis* (raw and cooked) was presented in Fig. 7. In all the starches, % transmittance was slightly reduced as the number of weeks of storage increased. Similar time-dependent reduction in % transmittance has been reported for banana starch (Bello-Perez *et al.,* 2000). This pattern is due to the fact that storage of gelatinized starch at low temperature produced retrogradation with the increase in the Resistant Starch (RS)content (Bello-Perez *et al.,* 2005). Chung *et al.* (2009) observed a similar decrease rapidly digestible starch level and increased slowly digestible starch and RS levels on high-moisture treatment, for gelatinized corn, pea and lentil starches.

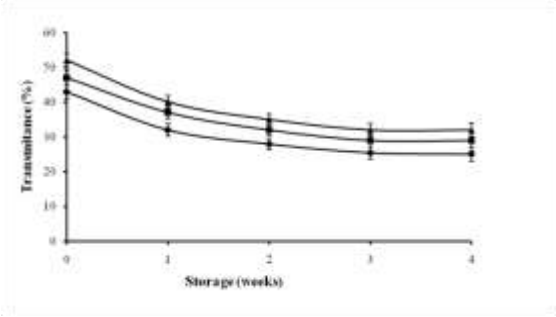


Fig. 7. Evolution of clarity of starch gels from raw (●), baked (¦) and boiled (▲) *Coulaedulis* seeds during storage at 4°C.

In vitro starch hydrolysis rate

Hydrolysis rates of $SEFCES_{ra}$, $SEFCES_{bo}$ and $SEFCES_{ba}$were shown in Fig. 8. The digestive juice of the snail *A. ventricosa* can act on both raw and cooked seed starches. This same pattern of granule degradation was also observed during in vitro digestion of wheat starch granules by larval *Tribolium castaneum* alpha-amylases (Baker *et al.,* 1994). Hydrolysis was occurred in the following phases: rapid hydrolysis (0-20 min), slow hydrolysis (20-140 min) leading to maximal hydrolysis after about 140 min. The hydrolysis of the remaining samples after 140 min was increasing at a very low rate. The SEFCES had high starch digestibility probably because of the small size of its starch granules (1 to 3 µm), low gelatinization temperature and limited content of resistant starch (Resio and Suarez, 2001; Bello-Perez *et al.,* 2006). Dietary fibre tends to reduce starch digestibility by trapping starch granules within cell wall structures or by directly hindering starch digestion and intestinal absorption of its degradation products that get caught in the viscous gel matrix of soluble dietary fibre (Wolever, 1990). Data suggest that the amount of soluble fibre in $SEFCES_{ra}$ is insufficient to affect the rate of starch digestion. The $SEFCES_{ra}$ was lower ($p<0.05$) digested than the $SEFCES_{bo}$ and $SEFCES_{ba}$. This result shows that the effect on hydrolysis rate was significantly ($p<0.05$) enhanced by cooking. This was consistent with the result obtained for potato and canna starch granules. The $SEFCES_{bo}$ showed a higher ($p<0.05$) hydrolysis rate than that of the $SEFCES_{ba}$. This pattern seems that the $SEFCES_{bo}$ are more gelatinized and partly solubilized than the $SEFCES_{ba}$. There is an increase in the RDS and slowly digestible starch content and decrease in resistant starch after cooking compared to raw seed. This explains the great improvement of starch hydrolysis attained after processing methods. In general, changes in starch hydrolysis rate or digestibility are correlated with the differences in disruption of crystalline regions due to the various processing methods (Englyst *et al.,* 1992; Mbofung *et al.,* 1999). The relative order of hydrolysis was $SEFCES_{bo}>SEFCES_{ba}>SEFCES_{ra}$.

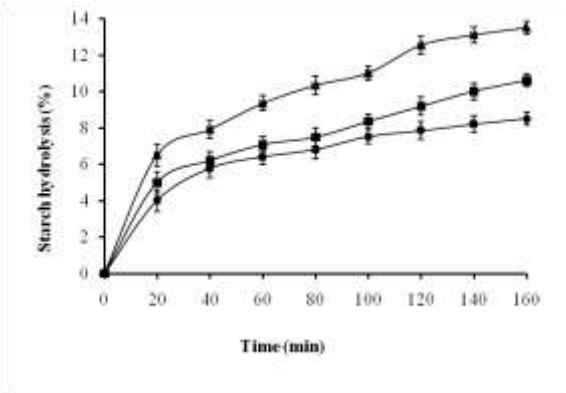


Fig. 8. Hydrolysis of starches extracted from raw (●), baked (¦) and boiled (▲) *Coulaedulis*seeds with the digestive juice of the snail *Archachatinaventricosa*.

Conclusion

The results obtained in the present work have shown that granules of $SEFCeS_{ra}$ were round in shape, with sizes ranging from 4.29-21.39 µm. Theydid not show unusual features of granules such as collapsed granule, cracked surface and some defects, but those of $SEFCeS_{bo}$ and $SEFCeS_{ba}$ showed critical morphological changes. The loss of starch granules integrity was greater in the boiled seeds. Also, boiling and baking of *C. edulis*seeds caused changes in the fat content, amylose content, swelling power, solubility, starch gel clarity and *in vitro*hydrolysis rate of starches. The starch of baked seeds was obviously less affected, compared to starch of boiled seeds. The low protein content (0.1%) of starches extracted from *C. edulis* would make them useful in the manufacture of highglucose syrups. The *C. edulis*starch's excellent clarity makes it potentially useful in sauces and in products such as fruit pie fillings and candies.

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